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In Situ Treatment of PCBs by Anaerobic Microbial Dechlorination in Aquatic Sediment: Are We There Yet?

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Abstract

The remediation of PCBs in soils and sediments remains a particularly difficult problem to solve. The possibility of *in situ* degradation by microorganisms has been pursued for many years since this approach has the potential to provide a cost-effective and environmentally sustainable alternative to dredging for treatment of PCB impacted sites. Because PCBs are hydrophobic and partition into organic material they accumulate in anoxic environments well poised to support anaerobic dechlorination of highly chlorinated commercial PCBs to congeners that are susceptible to complete aerobic degradation. Laboratory research over the past 25 years is now leading to new microbial technologies that could soon be tested for treatment of PCB impacted soils and sediments in the field.

Introduction

Polychlorinated biphenyls (PCBs) were manufactured as inert, stable, flame- and oxidation-resistant products for a variety of applications such as coolants and dielectric fluids in electrical equipment. Although their manufacture was banned in the U.S. in 1979 and subsequently worldwide in 2001, PCBs persist in the environment as a result of past disposal practices and accidents. Because PCBs are hydrophobic they partition preferentially to organic particles in the environment, which serve both as long-term reservoirs and as carriers that can distribute PCBs great distances from the original point source as a result of current and wind. Although sorbed PCBs resist migration into the water fraction, PCBs enter the food chain by ingestion and desorbtion in benthic microorganisms leading to eventual bioaccumulation and biomagnification of PCBs in organisms higher up in the food chain [1]. PCBs are listed as priority organic pollutants by the EPA (http://nlquery.epa.gov) due to the environmental impact and health risk that they pose and there has been a long search for cost-effective and environmentally sustainable methods such as bioremediation to treat them *in situ*.

Anaerobic Dechlorination

Discovery

Highly chlorinated PCBs common in many commercial Aroclors resist aerobic degradation until they are partially dechlorinated by anaerobic microbial dechlorination. The first evidence of anaerobic PCB dechlorination was based on changes in congener patterns observed downstream of a capacitor plant that released Aroclor 1242 into the Hudson River [2], which was attributed to microorganisms that could derive energy by using PCBs as electron acceptors; a process later termed dehalorespiration [3]. Quensen et al. [4] followed by others showed that microbial dechlorination of single PCB congeners and Aroclors could be reproduced in laboratory microcosms with PCB-impacted sediments from numerous sources ([5–6] and reviewed in [7–9]). Specific pathways and rates of PCB dechlorinating activity have been reported in freshwater, estuarine and most recently in marine sediments [10], and because they can vary greatly between sediments models have been developed recently to assist in predicting all potential dechlorination pathways for a specific site [11].

Identification and Growth in Culture of PCB Dechlorinating Bacteria

Identification of PCB dehalorespiring bacteria (Table 1 and Fig. 1) eluded investigators for a number of years because the microbes could only be grown in the presence of sediment or soil particles. Using a combination of selective enrichment in sediment microcosms and comparative sequence analysis of 16S rRNA genes after PCR amplification with universal primers, Holoman et al [12] first identified a phylotype within the Chloroflexi as the likely biocatalyst for PCB dechlorination. The identity of two PCB dechlorinating bacteria, strains o-17 and DF-1, were later confirmed in co-culture with *Desulfovibrio* spp. that were required for growth in a sediment-free medium [13–14]. These were the first reports of sustained anaerobic PCB dechlorination in the complete absence of sediment with PCBs serving as the sole electron acceptor and eventually led to the isolation of "*Dehalobium chlorocoercia*" DF-1 [15]. *Dehalococcoides mccartyi* strain 195 (previously *D. ethenogenes* [16]) has been shown to dechlorinate PCBs in the presence of chlorinated ethenes [17]. Later, *D. mccartyi* strain CBDB1 was demonstrated to dechlorinate a broad spectrum of PCBs in the absence of sediment [18]. Yoshida et al [19] reported reductive dechlorination of a tri- and tetra-chlorobiphenyl in a sediment-free consortium containing two phylotypes of *Dehalobacter*, but this activity has not yet been confirmed in pure culture. An alternative approach substituting silica powder for sediment has recently resulted in the sustainable growth of Aroclor-dechlorinating of microorganisms under sediment-free conditions [5,20]. A possible role of sediment in promoting reductive dechlorination could be to serve as a substrate for biofilm formation in close proximity to adsorbed hydrophobic PCBs. This conclusion is consistent with the formation of PCB degrading biofilms, described as "clay hutches", on sandy clay soil contaminated with PCBs and the observation that biofilms form on the surface and eventually invade PCB droplets in water [21–22]. The ability to culture PCB dechlorinating bacteria in sediment -free medium was a critical achievement for eventual mass culturing of inoculum for bioaugmentation.

In addition to a preference for solid substrates, some PCB dechlorinating bacteria require growth factors provided by other microorganisms. *Dehalococcoides* spp. often require acetate as a carbon source and cobalamin as a growth factor [16,23]. Growth and dechlorination by *D. mccartyi* 195 is also stimulated by an unidentified factor in sterile cell-free supernatant of *Dehalococcoides* enrichment cultures [16], but the addition of select amino acids will also stimulate growth and dechlorination rates [24]. *D. chlorocoercia*DF-1 and strain *o*-17 require coculturing with a *Desulfovibrio* spp. or addition of sterile cell-free culture supernatant from pure *Desulfovibrio* spp., however the nature of this growth factor has also not been identified [15]. *D. mccartyi* 195 has been shown also to more rapidly grow

and dechlorinate tetrachlorethene when grown in co-culture with *D. vulgaris* Hildenborough supplied with lactate as the sole carbon and energy source [25]. A sulfate reducer may supply growth factors such as amino acids and cobalamin (required for dehalogenases) as well as a slow release of electron donor to the dehalorespiring bacterium via inter-species hydrogen exchange.

Detection and *In Situ* Monitoring of PCB Dechlorinating Bacteria

PCB dechlorinating phylotypes are difficult to detect using universal 16S rRNA gene primers in nutrient-rich sediment because of their slow growth rates and low yields (<1%) relative to other indigenous species. *Dehalococcoides* sp. specific primers DHC1F/DHC1377R developed by Hendrickson et al [26] will detect PCB dechlorinating phylotypes within the *Dehalococcoides* spp. A complementary set of primers (Univ14F/Dehal1265R) developed by Watts et al. [27] will detect PCB dechlorinating phylotypes within the non-*Dehalococcoides* spp. including strain *o*-17 and *D. chlorocoercia*DF-1 within the Chloroflexi. A group-specific 16S rRNA gene primer set, Chl348F and Dehal884R, will concurrently detect both *Dehalococcoides* spp. and *o*-17/DF-1-like PCB-dechlorinating species in soils and sediments [28]. A limitation of currently available 16S rRNA gene primer sets is that the assays are presumptive since they do not differentiate augmented from indigenous species, which might include both PCB dechlorinating and any non-dechlorinating phylotypes. Since 16S rRNA gene sequences will not differentiate strains within this clade, primers need to be designed for highly conserved protein or nucleotide encoding genes with unique sequence not detected in the indigenous background. Park et al [29] developed primers for known and putative reductive dehalogenase (*rdh*) gene homologs that differentiated two *D. mccartyi*-specific gene sequences from a background of indigenous *Dehalococcoides* phylotypes in sediment microcosms bioaugmented with *D. mccartyi*. The development of additional strain specific primer sets for quantitative monitoring of bioaugmentation inoculum by qPCR should be feasible with the availability of genome sequences for three of the known PCB dechlorinating bacteria *D. mccartyi* strains 195 and CBDB1, *and D. chlorocoerocia,* or by identifying putative dehalogenases from other PCB dechlorinators using degenerate *rdh* primers [30].

From Microcosm to Mesocosm to Field Trials

Biostimulation

Biostimulation of indigenous PCB dechlorinating bacteria has been achieved by halopriming with halogenated aromatic compounds. Halopriming may increase the biomass of the dehalogenating microbial catalysts, induce genes required for dechlorination, and possibly support dehalorespiration or cometabolism of additional PCB congeners. Bedard et al [31] first described the stimulation of weathered Aroclor 1260 dechlorination in sediments by addition of 2,5,3′,4′-tetrachlorobiphenyl and subsequently showed that the same could be achieved with bromated biphenyl congeners (PBBs) [32]. Although PBBs were more effective stimulants that could be completely dehalogenated [33], the deliberate addition of relatively high concentrations (0.6–1 PPM) of halogenated biphenyls into the environment would be subject to regulatory scrutiny. Halogenated benzoates and other halogenated aromatic compounds can also prime PCB dechlorination but they are not as effective as PBBs [34–36]. Most recently, Park et al. expanded the list of haloprimers to include the fungicide pentachloronitrobenzene, which was demonstrated to stimulate more dechlorination of weathered PCBs than tetrachlorobenzene [29,36].

Biostimulation has also been observed after addition of a slow release electron donor. Addition of Fe^0 as a source of cathodic hydrogen stimulated the microbial dechlorination of selected PCB congeners in microcosms containing PCB-impacted Baltimore Harbor

sediment [37] and of Aroclor 1254 in a marine sediment [38]. Periodic addition of Fe^0 was observed to stimulate the indigenous population of *Dehalococcoides* in a microcosm study with PCB impacted sediments from Lake Hartwell, New Bedfod Harbor and Rosanna Marsh [39]. The low levels of hydrogen released by periodic replenishment with Fe^0 provided *Dehalococcoides* a greater competitive advantage over other hydrogen utilizers such as methanogens and sulfate reducing bacteria, but the effect of Fe^0 on PCB dechlorination activity in the microcosms was not reported. In contrast Fe^0 did not stimulate reductive dechlorination of PCBs in microcosms containing sediment from the Raisin River in Michigan unless they were bioaugmented with an actively dechlorinating culture, which suggests that biostimulation will not be effective in sites that lack a viable indigenous population of PCB dechlorinating bacteria [40]. Although biostimulation with Fe^0 has the potential to be an effective cost-effective treatment for *in situ* treatment of PCBs, with or without bioaugmentation, the effect of Fe^0 on dechlorination of weathered Aroclor-impacted sediments remains to be tested.

Electrochemical techniques have been used in the past to treat pollutants in groundwater or sediment [41]. The use of carbon cloth electrodes to supply electron donor and acceptor directly to microbes was recently demonstrated to stimulate the dechlorination of tetrachlorobenzene [42]. The method enables one to control the redox, hydrogen and oxygen supply to microorganism within electrochemically reactive sediment caps. Applying an electric current to sediment microcosms, Chun et al [43] recently demonstrated the removal of up to 60% (by mass) of weathered PCBs from Fox River sediment. This result was dependent on the action of anaerobic and aerobic microbes when voltage exceeded 2.2V and H_2 and O_2 were generated. However, degradation was most apparent in the absence of electrolytic O_2 generation with 1.5V applied, suggesting an expanded role for anaerobes in the degradation of the PCBs.

Bioaugmentation

Another potential approach for *in situ* treatment of PCBs is bioaugmentation with dehalogenating microorganisms. Bedard et al. [44] observed in an enrichment culture that a critical mass of cells was required before reductive dechlorination of spiked Aroclor 1260 was detected and proposed that low indigenous numbers of dehalorespiring bacteria explains why substantial attenuation of PCBs is rarely observed in the environment. However, there have been very few studies to date describing anaerobic bioaugmentation with PCB dechlorinating isolates to stimulate *in situ* treatment of Aroclor-impacted sediments. May et al. [15] showed that bioaugmentation with DF-1 stimulated the reductive dechlorination of weathered Aroclor 1260 (4.6 ppm) in contaminated soil microcosms, and Krumins [36] reported that the addition of *D. mccartyi* and pentachloronitrobenzene stimulated the dechlorination of weathered Aroclors 1248, 1254, and 1260 (2.1 ppm) in sediment microcosms. More recently Payne et al [45] demonstrated 56% reduction (by mass) of total penta- and higher chlorinated PCBs in open mesocosms containing weathered Aroclor 1260 (1.3 ppm) after bioaugmentation with *D. chlorocoercia* DF1, which was sustained within the indigenous microbial population after 120 days. These combined studies provide the most convincing evidence to date that using bioaugmentation for *in situ* treatment of weathered PCBs is potentially feasible.

Coupling Anaerobic PCB Dechlorination with Aerobic Degradation

Extensive dechlorination of Aroclor 1260 has been observed by the complementary activities of three member consortia in sediment microcosms [46] and with an individual isolate, *D. mccartyi* CBDB1, in sediment-free culture [18]. As early as 1995 it was recognized that the anaerobic dechlorination of more highly chlorinated congeners followed by the aerobic degradation of those dechlorination products was occurring in the

environment [47], and this was suggested to be a potential treatment strategy for PCB impacted sediment. Several investigators have demonstrated that sequentially treating PCB impacted sediment in an anaerobic PCB dehalorespiring enrichment followed by transfer in an aerobic culture containing *B. xenovorans* LB400 effectively degraded Aroclors by as much a 70% [48–49]. However, all sequential anaerobic-aerobic studies to date have been conducted in closed microcosms and do not represent *in situ* conditions. One current limitation of this approach is that Aroclors contain varying percentages of congeners with tri- and tetra *ortho* CBs that are recalcitrant to aerobic degradation. Since reductive dechlorination of *ortho*-chlorines has been reported infrequently in the environment [32], *in situ* treatment of a PCB impacted site might require bioaugmentation with an *ortho*-dechlorinating microorganism in order to prevent a build up of recalcitrant *ortho*-PCBs. Fagervold [50] reported that addition of the strain *o*-17 in co-culture with other PCB dechlorinating microorganisms reduced the accumulation of *ortho*-CBs in sediment microcosms. Sequential bioaugmentation by anaerobic dechlorination with a consortium containing strain *o*-17 and aerobic degradation with recombinant strains such as *Burkholderia xenovorans* LB400 (*ohb*), which effectively grows on and mineralizes *ortho* substituted PCBs [51], has the potential to lead to more complete degradation of Aroclors.

In situ treatment of PCBs – from laboratory to field

In situ treatment will require sufficient scale-up of biomass to bioaugment large areas of impacted sediment. Payne et al [45] showed that approximately 10^5 cells g^{-1} (wet wt) sediment provided a sufficient critical mass of cells to effectively stimulate dechlorination of weathered Aroclor 1260. Based on this cell density and the assumption that bioamendment applied to the top cm will be distributed deeper into the sediment by bioturbation, one km^2 of PCB impacted sediment would require 10^{15} cells grown in a culture volume of 10,000 l and maximum cell density of 10^8 cells ml^{-1}. Although large-scale culturing of *Dehalococcoides* sp. grown on chloroethenes has been reported in volumes up to 3,200 l [52], bioaugmentation of dechlorinating species grown at large scale with PCBs would restrict their distribution in the environment. Thus far the only electron acceptors known to support growth of PCB dechlorinating bacteria are halogenated aliphatic or aromatic compounds that are also considered persistent organic pollutants. Unless a non-toxic electron acceptor is identified, methods need to be developed for one that can be readily removed from the cells. Miller et al [53] reported that *D. chlorocoerocia* pre-grown with tetrachloroethene showed no significant lag in growth when transferred to 2,3,4,5-tetrachlorobiphenyl, which suggests that residual volatile substrates such as chlorinated ethenes could be sparged from cultures prior to harvesting. Alternatively, substituting more readily used electron acceptors such as PPBs might be a viable approach for application in the field.[Bedard 1998]

A suitable means for deploying PCB dechlorinating bacteria in the field is also required. Unlike more soluble organohalides such as chloroethenes, which can be bioaugmented by pumping microorganisms and nutrients into groundwater, PCBs are hydrophobic and tend to become immobilized by adsorption to soil and sediment particles. Effective bioaugmentation of PCB impacted soils and sediments will require a method for inoculating sediment either by direct injection or deployment on solid particles. Dehalogenating microorganisms enriched in microbial granules have been proposed as a mean for deployment in sediments [54–55]. Payne et al [45] recently showed that bioaugmentation of sediments contaminated with weathered Aroclor 1260 was equally effective either by direct injection or on GAC particles. Organic particles such as clay or GAC would strongly sorb PCBs in an aqueous environment and provide substrate for biofilm formation in close proximity to the hydrophobic PCBs [21–22]. The ability to use a solid substrate such as clay or GAC particles for inoculation of cells offers a possible solution for dispersing cells in the field.

Conclusion

Currently the predominant treatment option for PCBs in sediments is dredging followed by stabilization by dewatering and landfilling, but this approach is environmentally disruptive and unsustainable. Passive capping limits exposure of PCBs to the food chain, but since PCBs remain in the environment a potential long-term risk due to gradual or acute disruption of the cap remains. Development of a tractable microbial *in situ* treatment system would provide a cost-effective, and environmentally sustainable alternative to dredging by reducing the health risks associated with sediment disruption, reducing overall energy use, effectively negating the requirement for extensive waste management and obviating the requirement for substantial habitat restoration. Over the years, several anaerobic bacteria with a broad range of PCB dechlorinating activity have been described and show great potential to be coupled with aerobic PCB degrading bacteria. Novel means of supplying electron donor to the dechlorinators and electron acceptors, methods to mass culture and harvest PCB dechlorinators, design of molecular tools for monitoring the fate of inocula, and approaches for field deployment are currently under development. While much remains to be done to develop methods to advance degradation further, may of the critical components are in place to begin field trials and optimize this biotechnology for effective *in situ* treatment in PCB-impacted environments (Fig. 2).

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•• of outstanding interest

• of special interest

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Highlights

- Microbial catalysts with different PCB dechlorinating activities are cultured
- Biostimulation and bioaugmentation has been successful in the laboratory
- Molecular tools for monitoring dechlorinating bacteria *in situ* are available
- Methods to deploy these catalysts in the field are currently under development

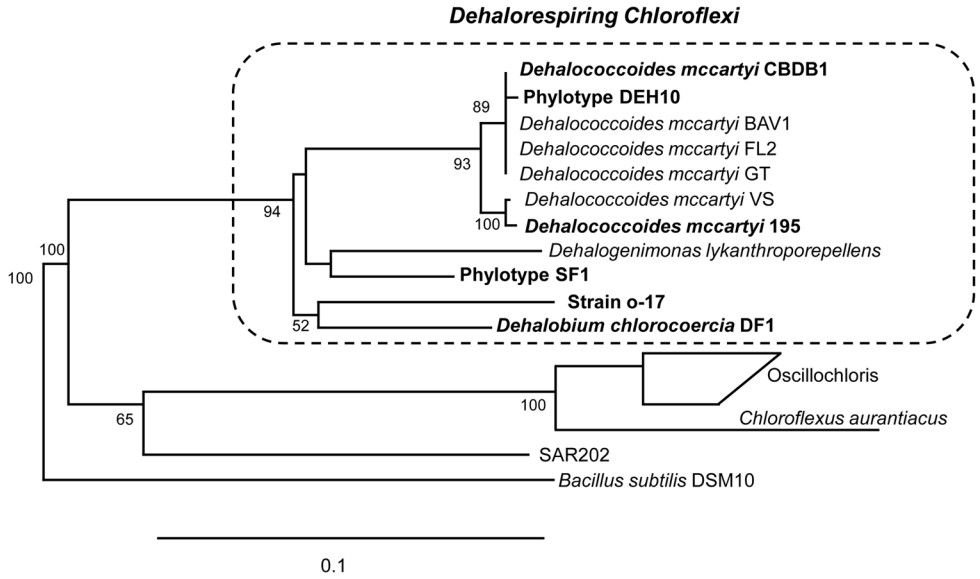

Figure 1.

Phylogenetic tree showing the relationships between confirmed PCB dechlorinating bacteria and phylotypes (**Bold**) and other species within the dechlorinating Chloroflexi group based on comparative sequence analysis of 16S rRNA genes Bootstrap values over 50 are indicated at the branch points. The scale bar indicates 10 substitutions per 100 nucleotide positions.

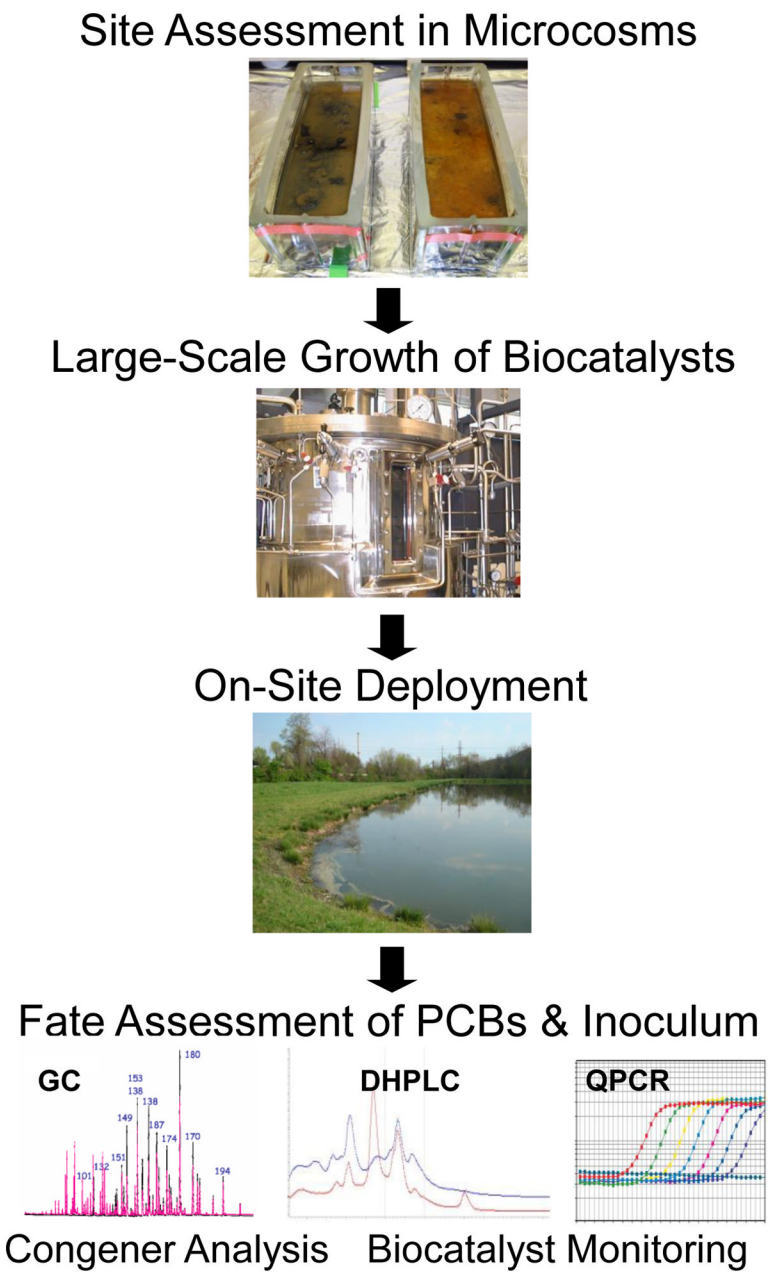

Figure 2.
Proposed scheme for *in situ* treatment of PCB impacted sediments using biaugmentation.

Table 1

Dehalorespiring bacteria and phylotypes with confirmed PCB dechlorinating activities

Strain or phylotype	Electron donor	Dechlorination activities	Culture status	Reference
"*Dehalobium chlorocoercia*" DF-1	H_2, formate	Double flanked *meta/para*	Isolate	Wu 2002
Strain *o*-17	Acetate	Flanked *ortho/meta*	Co-culture	Cutter 2001
Phylotype DEH-10	Unknown[a]	Double flanked *meta/para* *Para* flanked *meta*	Sediment microcosm	Fagervold 2005, 2007
Phylotype SF-1	Unknown[a]	Double flanked *meta* *Ortho* flanked *meta*	Sediment microcosm	Fagervold 2005, 2007
Dehalococcoides sp. CBDB1	Hydrogen	Double and single flanked *para* Double flanked *meta*	Isolate	Adrian 2009
Dehalococcoides mccartyi 195	Hydrogen	Double flanked *meta/para*	Isolate	Fennell 2004

[a]Grown with a mixture of acetate, propionate, butyrate

[b]Specific activities of individual phylotypes not determined